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HAMA, JOANNE	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/538,736	BRANCACCIO ET AL.	
	Examiner	Art Unit	
	Joanne Hama, Ph.D.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 June 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-25 and 40-42* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-25 and 40-42* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant filed a response to the Non-Final Rejection of March 27, 2007 on June 27, 2007.

Claims 26-39 are cancelled.

Per the Petition Decision of December 11, 2007, wherein the lack of unity determination has been withdrawn, claims 10, 20-23 are now included in the prosecution. It is noted that the limitations, as recited in claim 5, will also be examined.

Claims 1-25, 40-42 are under consideration.

Withdrawn Objection/Rejection

Claim Objection

It is noted that per the Petition Decision of December 11, 2007, wherein the lack of unity determination has been withdrawn, the objection to the claims as they are drawn to non-elected subject matter is withdrawn.

Claim Rejections - 35 USC § 101

Applicant's arguments, see pages 6-15 of Applicant's response, filed June 27, 2007, with respect to the rejections of claims 1-9, 11-19, 24, 25, 40-42 have been fully considered and are persuasive.

Applicant indicates that melusin null mice develop cardiac dilation and then heart failure when subjected to chronic conditions of high blood pressure as exemplified by surgical banding of the transverse aorta. Under these conditions

and in the absence of melusin, the heart rapidly undergoes dilation and congestive heart failure. This pathology and phenotype of the animal model closely mimic the clinical histories of human patients with hypertension, who frequently develop cardiac dilation and congestive heart failure. The fact that the melusin null mouse model is highly relevant for human pathology is also demonstrated by a study of human patients who have developed heart failure as a consequence of aortic stenosis (Brokat, 2007). In these patients, in fact, reduction in melusin expression parallels the functional cardiac impairment. These data strongly indicate that reduced expression of melusin in the heart corresponds to increased susceptibility to develop heart dysfunction and, on the other hand, indicates that therapeutic strategies aimed at sustaining melusin function will represent a highly promising new treatment for humans. In response, Applicant's assertion that the claimed mouse is a model of a human condition is found persuasive and the rejection as it applies to this issue is withdrawn. As such, the rejection as it applies to claims 1-9, 11-19, 24, 25, 40-42 is withdrawn.

New/Maintained Rejections

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9, 11-19, 24, 25, 40-42 remain rejected and claims 10, 20-23 are newly rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for

1) a transgenic mouse comprising a homozygous disruption of a gene encoding melusin in its somatic and germ cells, wherein said mouse, following a hypertensive condition, exhibits a phenotype selected from the group consisting of: impaired heart hypertrophy, heart dilation, and heart failure,

2) a method of producing a mouse homozygous for a targeted disruption in melusin, wherein said targeted disruption inhibits production of wild type melusin, comprising the steps of:

(a) injecting a mouse embryonic stem cell comprising said targeted disruption produced by homologous recombination in to a blastocyst;

(b) implanting said blastocyst into a pseudopregnant mouse;

(c) allowing said blastocyst to develop to term to produce a chimeric mouse;

(d) breeding said chimeric mouse, wherein said chimeric mouse is capable of transmitting said targeted disruption, to produce a heterozygous mouse; and

(e) breeding said heterozygous mouse to produce said homozygous mouse,

3) method of using said transgenic mouse and cells obtained from said transgenic mouse in methods of studying heart pathology, and

4) method of using said transgenic mouse and cells in screens for compounds of pharmacological activity,

does not reasonably provide enablement for:

1) a non-human transgenic animal and cells obtained from said animal having any altered melusin expression,

2) a non-human transgenic animal having an inactivated melusin gene, wherein said animal develops, independent of a hypertensive induction, a phenotype selected from the group consisting of: impaired heart hypertrophy, heart dilation, and heart failure,

3) methods of using said non-human transgenic animals.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Given the Petition Decision of December 11, 2007, the Examiner provides the following analysis to address the rejections of all embodiments encompassed in claims 1-25, 40-42. Response to Applicant's rebuttals of June 27, 2007 will be provided following the new issues of rejection.

Claim 1 is readable such that the non-human transgenic animals encompassed by the claimed invention exhibit overexpression and decreased expression of melusin. At the time of filing, the art teaches that there was unpredictability in arriving at transgenic overexpression animals. Franz et al., 1997, J. Mol. Med., 75: 115-129 teach that an artisan may encounter limitations and difficulties as unexpected results or no effects in the transgenic phenotype.

The choice of animal species and unexpected functions of the candidate gene or compensatory alterations of other genes may contribute to these phenomena (Franz et al., page 116, 1st col., 3rd parag.). In another example, the art teaches that there is unpredictability in generating transgenic non-human animals wherein the same transgene construct used in mice and rats generated a phenotype in rats, but did not generate a phenotype in mice. Hammer et al. 1990, Cell, 6: 1099-1112 created both transgenic mice and rats expressing the human HLA-b27 gene and beta-2 microglobulin. Although both transgenic animals bearing the HLA-b27 gene expressed the gene, transgenic mice did not show any HLA-b27 associated disease, whereas the transgenic rats demonstrated most of the HLA-b27 related diseases (Hammer, et al., page 1099, col. 2, lines 20-28). This shows that the integration of a transgene into an alternative species may result in widely different phenotype responses. In addition to teaching that transgene constructs are unpredictable, the art teaches that the components of the transgene construct are unpredictable in transgenic non-human animals. The art teaches that promoters are unpredictable in transgenic non-human animals. Cowan et al. 2003, Xenotransplantation, 10: 223-231 teach that promoters of three human genes, ICAM-2, hCRPs, and PECAM-1, are predominantly expressed in vascular endothelium in mice and pigs. When tissue specific expression was measured, it was found that while mice showed a distinct expression profile of the three human genes, the tissue expression profiles of the three human gene promoters were distinctly different in pigs. The authors concluded that "promoter performance in mice and pigs was not equivalent," and

that "the weak expression driven by the human ICAM-2 promoter in pigs relative to mice suggests the need for additional regulatory elements to achieve species-specific gene expression in pigs (Cowan et al., abstract)." In the case of expressing heterologous genes of interest, the art teaches that proteins encoded by an artisan's gene of interest do not always behave in a predictable manner in heterologous non-human animals. For example, Hammer et al. 1986, J. of Anim. Sci., 63: 269-278 teach that while transgenic mice that overexpressed human growth hormone exhibited enhanced growth, transgenic pigs that expressed human growth hormone did not increase weight gain (Hammer et al., page 276, under "Effect of Foreign GH on Growth"). As these issues apply to the instant invention, an artisan cannot predict what phenotype(s) an overexpression melusin mouse will exhibit, nor can an artisan predict that overexpression of melusin results in any phenotype that is related to any disease or disorder such that the claimed animals can be used. Thus, because neither the specification nor the art provide any guidance for the use of any transgenic overexpression melusin non-human animal, an artisan is not enabled to use such non-human animal.

With regard to the claims being drawn to the claimed non-human animal overexpressing antisense RNA, the art at the time of filing teaches the unpredictability in arriving at transgenic animals that express antisense. While it is understood that antisense is a way to phenocopy the phenotype seen in knockout animals, the art teaches that skepticism regarding the validity and utility of antisense technology remains. Contributing to this uncertainty is the lack of

systematic studies exploring antisense oligonuclotide use in vivo and the many technical and methodological challenges intrinsic to the method (Stone and Vulchanova, 2003, Advanced Drug Delivery Reviews, 55: 1081-1112, abstract). Stone and Vulchanova teach that many groups have invested resources in antisense oligonucleotide (ASO)-based experiments and have been frustrated by uninformative, uninterpretable, misleading, or simply irreproducible results. What contributes to this frustration is attributed in large part to the plethora of technical issues that must be addressed when designing an ASO-based experiment. Among the critical issues are sequence activity and selectivity, oligonucleotide stability, proper use of controls, route of administration and uptake, dose-dependence, time-course and adequate evaluation of knockdown (Stone and Vulchanova, page 1087, 2nd col. under "5. Experimental design of in vivo antisense-based studies" to page 1088).

One major factor influencing the success of antisense treatment is the identification of active ASO sequences. Estimates suggest that between 5-15% of randomly selected ASOs will be sufficiently active at a target gene to generate meaningful results. This is largely due to the inaccessibility of most of the RNA molecule for ASO binding as a consequence of its complex secondary structure. Furthermore, associations with cellular proteins will render additional regions unavailable for binding. While there are methods that increase the probability of finding active sequences, the current state of knowledge is such that at some level, sequence identification remains a hit or miss process (Stone and Vulchanova, page 1088, 1st col., 1st parag. under "5.1 Antisense oligonucleotide

sequence selection"). In addition to this, Stone and Vulchanova teach that in vitro efficacy does not necessarily predict nor guarantee in vivo efficacy. The RNA structure, the complement of accessory proteins and the regulation of the target gene may be different in vivo than in the in vitro system of screening for active antisense. Further, the percent activity demonstrated in vitro might not predict the percent knockdown in vivo (Stone and Vluchanova, page 1089, 1st col., 2nd parag.).

Another major factor that influences the success of antisense is sequence selectivity. While part of the allure of antisense comes from claims of absolute selectivity, there is surprisingly little evidence to support this contention. Rather, the art indicates the effect of mismatches (substitution of bases that are not complementary to the target RNA) on the activity of ASO. While an intact 25-mer produced 79% degradation of the target mRNA, sequences with 17/25 and 14/25 matches resulted in 32 and 37% degradation respectively, an unrelated control produced 13% degradation. The art teaches that as few as 10 consecutive complementary bases are sufficient to produce cleavage of a target RNA and this is not prevented by mismatched flanking sequences (Stone and Vulchanova, page 1090, 1st parag. under "5.2 Antisense selectivity"). It is noted that a similar problem occurs with siRNA (see Jackson et al., 2003, Nature Biotechnology, 21: 635-637, abstract). As these issues apply to the instant invention, nothing in the specification addresses what steps are required to arrive at melusin antisense molecules that have activity and that are specific for melusin. Further, in light of the teachings of making overexpression melusin transgenic animals, the

specification does not provide guidance as to what promoter(s) are required such that antisense melusin is expressed at levels such that a phenotype is seen. It is noted that the art teaches that partial reduction in mRNA levels do not necessarily result in animals that exhibit a phenotype (Sokol and Murray, 1996, Transgenic Research 5: 363-371, Table 1, see for example, the HPRT mouse). Also, the art teaches that different expression levels can also result in unexpected phenotypes (Storm et al., 2003, PNAS, USA, 100: 1757-1762, abstract). As such, the specification does not provide guidance that expression of an antisense melusin construct necessarily will produce an animal model that phenocopies the knockout melusin mouse.

In addition to the above problems associated with a transgenic animal that comprises a stable expression construct in its genome, animals treated transiently with antisense RNA and DNA have additional problems. In addition to exogenous antisense RNA and DNA, the claims encompass expression vectors (e.g. plasmids) as well. It is noted at this point that claim 5 is drawn to the use of "DNA interference". The specification does not provide any guidance as to what is meant by this term. Lin and Ying, 2001, Current Cancer Drug Targets, 1: 241-247 use "DNA interference" in the same context as "DNA antisense." As such, DNA interference has been interpreted to be the same as "DNA antisense."

With regard to the claims being readable on administration of a transgene construct or any exogenous antisense RNA or DNA, the route of administration raises issues of enablement. According to the art, in order for a carrier system to preferentially accumulate at sites of systemic disease, such as tumors, sites of

inflammation, and sites of infection, the carrier must exhibit long circulation lifetimes following intravenous injection. However, most gene delivery systems including viral vectors as well as non-viral vectors (e.g. lipoplexes, polyplexes, and lipopolyplexes) are rapidly cleared from circulation and are preferentially taken up by the "first-pass" organs such as liver, lung and spleen (Fenske et al., 2001, Current Opinion in Molecular Therapeutics, 3: 154-158, abstract). Further, Lowenstein and Castro, 2004, Current Opinion in Pharmacology, 4: 91-97, see abstract, teach that the choice of vectors, transgenes, regulatory elements, delivery approaches, and the capacity to transduce the appropriate target cell type influence the effectiveness of gene therapy. Further, even if the strategy is effective in experimental animals, issues relating to side effects of gene therapy, longevity of the transgene expression and diffusion throughout the tissue of interest limits the clinical potential of gene therapy. It is noted that while Lowenstein and Castro discuss the transgene in the context of gene therapy, the issues Lowenstein and Castro are similar to using transient gene expression constructs to arrive at an animal that exhibits a phenotype following transgene expression. As the teachings of Lowenstein and Castro apply to the instant invention, the specification provides no guidance that addresses how to accumulate expression vector in the cardiac tissue (how much transgene, how often is the transgene administered) such that upon expression of the transgene of interest and induction of a hypertensive condition, the claimed animal exhibits a heart disorder. In addition to this, it is unclear, based on the specification, how long an artisan would need to express the transient transgene construct before

the animal model is induced with a hypertensive condition, such that the claimed animal exhibits a heart condition. In a similar vein, with regard to administration of exogenous antisense RNA or DNA, the specification does not provide guidance of any dosing regimen that is used to arrive at the claimed animals. As such, with regard to the claims being drawn to "transient modification" of melusin expression in an animal, the specification does not provide an enabling disclosure of how to arrive at such animals such that they exhibit a phenotype and can be used as a model of disease.

With regard to administering RNA antisense and short interfering RNA (siRNA), the art teaches that antisense RNAs are rapidly degraded in serum in vitro, in biological fluids, and by most cells Oekelen et al., 2003, Brain Research Reviews, 42: 123-142; page 130, 1st parag. under "5.2 Type of chemical modification"). While the art teaches that antisense RNA can be chemically modified, problems associated with chemically modified RNA include binding to proteins and hence alter protein function (Oekelen et al., page 130, 2nd col., 2nd parag.). In addition to this issue, the art teaches that dosage of administered antisense must also be considered. Oekelen et al. teach that the number of transcripts to be inhibited by antisense depends on whether the mRNA to be blocked is a rare transcript or one that is abundant and whether the extracellularly administered antisense is in the relevant cell compartment (Oekelen et al., page 133, 2nd col., under "AO dosage"). Further, reiterating the problems, as discussed above, with regard to administration, Oekelen et al. indicate that an often-overlooked factor for functional studies involving antisense

oligonucleotides is the difference in time frame of protein reduction and biological effect. Depending on the expression level, half-life, turnover rate, and inducibility of the gene of interest, it may take considerably longer than one or two half-lives of the protein of interest to deplete cells of the protein of interest (Oekelen et al., page 134, 2nd col., under "6.5 Time course of administration"). As such, Oekelen et al. teach that there is unpredictability in voiding the cell of the protein of interest such that a loss-of-function cell type can be obtained. As these issues apply to the instant invention, it is unclear what antisense molecules of melusin are envisioned to be used to reduce expression of melusin mRNA, how much, when, and how often melusin antisense is to be administered to the host animal such that melusin protein levels is reduced, such that an artisan can induce a hypertensive condition such that a heart condition is seen. Given the unpredictability making exogenous antisense and issues of administration, an artisan is not enabled to practice the claimed invention for exogenously administered antisense.

Claims 12-14 are rejected because nothing in the specification teaches that any transgenic animals comprising a construct that overexpresses melusin or any construct that reduces expression of melusin spontaneously results in animals that exhibit the phenotypes listed in claims 12-14 (specification, page 13, lines 23-25). Rather, the phenotypes, as described in claims 12-14 are exhibited when mice comprising a gene disruption construction in their endogenous melusin gene are exposed to pressure overload (specification, page 14, lines 15-17). It is also noted that Applicant's response, June 27, 2007, page 12, also

indicates that the phenotypes are seen following chronic conditions of high blood pressure. As such, the specification does not provide guidance for an artisan to arrive at transgenic animals that overexpress melusin or exhibit a reduction in melusin expression, wherein said animal spontaneously exhibits a cardiac condition.

Applicant's arguments, see pages 15-21, filed June 27, 2007, with respect to the rejection of claims 1-9, 11-19, 24-25, 40-42 have been fully considered and are persuasive in part.

Applicant addresses the issue regarding the unpredictability in making transgenic animals. With regard to the teachings of Doetschman, 1999 and Jaenisch, 1988, Applicant indicates that Doetschmann and Jaenisch's teachings are contrary to what is indicated in the Office Action, page 12 (Applicant's response, pages 15-17). In response, the Examiner withdraws the rejection in part for the following reason. Regardless of whether there is (or not) an in vivo relationship between melusin and the phenotype, the bottom line is that the melusin knockout mice exhibit a phenotype that is a model of human condition. In this respect, the rejection is withdrawn. However, the rejection remains in light of the fact that the claims are drawn to transgenic animals having an altered melusin expression (including overexpression) and exhibit any phenotype (other than the heart condition described in the specification) and no phenotype. It is noted that the claims are not limited to knockout animals and encompass overexpression melusin animals and animals with reduced expression of melusin. While Applicant points to Doetschman, page 137, indicating that:

> The conclusion will be that the knock out phenotype do in fact provide accurate information concerning gene function that we should let the unexpected phenotypes lead us to the specific cell, tissue, organ, culture, and whole animal experiments that are relevant to the function of the gene in question, and that the absence of phenotype indicate that we have not discovered where or how to look for a phenotype,

figuring out where to look for a phenotype in a transgenic animal that does not appear to have one does not enable an artisan to use the claimed animal. Rather, it is undue experimentation for an artisan to look for an unobvious phenotype without any guidance. It is also undue experimentation for an artisan to determine a relationship between any phenotype and a disease or disorder such that the transgenic animal can be used. In addition to this, while Doeteschman's statement indicates that knockout mice can give insight regarding gene function, the statement is not indicative that knockout mice are models of disease. Rather, gene function is given in context of the host animal. Racay, 2002, Bratisl Lek Listy, 103: 121-126, teaches that:

> "mutations of some genes led to phenotype showing severe defects, which did not correspond to any clinically important disorder, indicating either high *in vivo* stability of the gene or the interspecies differences. From the view of human medicine, the differences among the species (it means the differences in genetic background, gene expression, metabolism, and signal transduction) represent the main limitation of the use of genetically modified animals as models of human diseases. Therefore some results acquired by this approach can not be applied in human medicine because of the differences between rodents and human beings (Racay, page 124, under point 5)."

Along the same vein, Jakel et al., 2004, Nature Reviews: Genetics, 5: 136-144 provides examples of transgenic mice wherein species-specific differences

causes problems in generating a human model of disease. In the case of making the Huntington's disease model, Jakel et al. teach that part of the difficulty in making a mouse model likely stems from the species differences of mouse and humans. These species differences include a rodent's basal ganglia is less vulnerable than its human counterpart, and that basic cellular biology, such as post-translational modification, is different from humans (Jakel et al., page 137, 2nd col., 3rd parag.). As such, the teachings of Doetschman, in view of Racay and Jakel et al., provide biological insight about the function of a gene of interest in the host animal, in which the transgenesis occurred. However, this is not indicative that every knockout or overexpression mouse is a model of human disease.

Applicant refers to the teachings of Jaenisch, page 1468, for indicating that specific mutations can be introduced into transgenic mice and that it is feasible to generate precise animal models and thus contradicts the Examiner's assertion that phenotypes in transgenic animals are unpredictable (Applicant's response, page 16). In response, Jaenisch's statement indicates the potential of what can be accomplished with transgenic animals. However, that does not change the fact that the art teaches that there are many examples of unpredictability in arriving at transgenic animals that exhibit a phenotype and that exhibit a phenotype related to a disease or disorder (e.g. see Kuehn, et al., 1987 and Jacks et al., 1992, previously cited). As such, while Examiner indicates an enabled scope, the claims encompass non-enabled embodiments and in that respect, the claims remain rejected.

With regard to Applicant's response of Moens, wherein Moens teaches that two different phenotype occurs in two different types of mutations, one null, the other leaky, Applicant indicates that two mutations would of course lead to two different phenotypes. Applicant indicates that one of skill in the art knows that is it is absolutely necessary to demonstrate the absence of protein expression to prove the efficacy of the mutation introduced by homologous recombination, as Applicant demonstrates in Figure 4. Applicant indicates that null mutations do not require undue experimentation (Applicant's response, pages 16-17). In response, while Applicant provides a response regarding null mutations, the claims are not so limited. As indicated above, the claims encompass transgenic animals that overexpress melusin and animals that express antisense RNA to melusin mRNA. In addition to Moens et al., Storm et al., 2003 teach that transgenic animals that overexpress a gene of interest or have expression of their endogenous gene partially reduced, result in animals with an unexpected phenotype and result in animal that exhibits a phenotype that is not related to a disease or disorder such that the animal can be used. As such, the rejection as it applies to this issue remains.

With regard to Applicant referring to the teachings of Wheeler, 2001, wherein on page 1351, Wheeler teaches that ES cells from various species of animals other than mouse have been made and that Prelle, 2002 teach on page 176 that ES cells in other species of animals extend to the germline such that lines of animals can be made, neither Wheeler nor Prelle teach that this is the case. Wheeler teaches that ES cells have been made; however, nothing in

Wheeler teaches that these ES cells extend to the germline. With regard to Prelle, Prelle indicates that nuclear transfer "circumvents" the need to make chimeric animals. As such, this is not indicative that chimeric animals can be made from ES cells obtained from animals other than mice. The rejection as it applies to the enabled scope being limited to ES cells from mice is maintained.

Applicant indicates that nuclear transfer technology is widely used in numerous laboratories to produce animals from the somatic cells of animal fetuses and adults. Applicant indicates that the generation of transgenic animals with nuclear transfer technology is based on the ability to introduce targeted gene mutations in somatic cells used for the nuclear transfer. Such ability is illustrated by McCreath, 2000 and Denning, 2001 (who teach sheep), and Lai, 2002 and Dai, 2002 (who teach pigs) (Applicant's response, pages 18-20). In response, while the art provides several examples of nuclear transfer, the art also teaches that nuclear transfer is not routine in the art. Oback and Wells, 2002, Cloning and Stem Cells, 4: 147-168 review the state of the art for donor cells used in cloning and state, "currently, we do not know what makes a good donor cell. In mammals, more than 200 distinct cell types are plainly distinguishable by morphology and more will probably be discovered when better molecular markers become available. Less than 5% of these have been tested as nuclear donors, and they all support development to blastocysts; however, many repeatedly failed to generate viable offspring (Oback and Wells, page 147, 2nd col., 1st parag.)." Oback and Wells further support the lack of teachings provided in the art with regard to donor cells that predictably result in live offspring by

showing that in different animal species, different somatic donor cells have been tested with varying results. For example, Wakayama and Yanagimachi tested eight cell types in nuclear transfer (NT) methodology in mice, and found that live offspring were obtained from fibroblast, undefined fetal gonadal and cumulus cells. Further, Kato et al. tested somatic donor cells in cattle and found that all supported development to blastocysts but live offspring were obtained from cumulus, oviduct, skin and liver cells (Oback and Wells, pages 155-156). Further, Oback and Wells teach that deciding which cell to use as a donor cell in NT methods is not clear because the cells that have worked in certain species are not the same cells that work in other species, and that they are often dissimilar in their cell cycle stage and their cloning competence. Oback and Wells provide a summary of cloning efficiencies from various somatic donor cells (see Table 1). It is noted that different cell types provide different cloning efficiencies with regard to different animal species. Thus, when taken with the specification's lack of teachings or guidance to enable the full breadth of the claimed invention (of any somatic cell donor) and the state of the art's clear teaching of the unpredictability of using any somatic cell as a donor in NT methodology, and the unpredictability amongst species of animals in using different somatic cells, an artisan could not reasonably arrive at the claimed invention.

The unpredictability in the NT art is further supported by the post-filing art of Campbell et al., 2005, Reprod. Dom. Anim.,40: 256-268. Campbell et al. review the state of the art of NT, and particularly, with regard to the choice of a

particular, suitable donor cell, they teach that although different cultured cells, as well as some somatic cells can be used in NT, there are varying results using these cell types, and they state that, "unfortunately no conclusion can be made on what is the most appropriate cell type for SCNT (Campbell et al., page 261, see under, Selection and culture of a suitable donor cell)." Tian et al. 2003, Reprod. Bio. & Endocrin., 98: 1-7, also support the unpredictability in selection of an appropriate donor cell, they teach that somatic cells have varying cloning competence and that although specific cell types have found to be successful in producing cloned animals, "A clear consensus, however, has not been reached as to the superior somatic cell type for nuclear transfer." They compared various donor cell types from the same donor animal and conclude that the donor cell type can significantly affect embryo development, both *in vitro* and *in vivo* (Tian et al, pages 3-4, under, Cloning competence of various somatic cell types). Thus, specific guidance must be provided to enable the claimed invention in view of the unpredictable state of the art with regard to NT in general, and specifically, for the specific donor cell used. For example, Li et al., 2003, Reprod. Bio. & Endocrin., 84: 1-6, state that, "overall efficiency of nuclear transfer is still very low and several hurdles remain before the power of this technique is harnessed. Among these hurdles include an incomplete understanding of biologic processes that control epigenetic reprogramming of the donor genome following nuclear transfer. Incomplete epigenetic reprogramming is considered the major cause of the developmental failure of cloned embryos and is frequently associated with the disregulation of specific genes. At present, little is known about the

developmental mechanism of reconstructed embryos. Therefore, screening strategies to design nuclear transfer protocols that will mimic the epigenetic remodeling occurring in normal embryos and identifying molecular parameters that can assess the developmental potential of pre-implantation embryos are becoming increasing important (Li et al., abstract)." Li et al. further state that, "The factors involved in the success of NT are very complex. Although many protocols have been modified and utilized in the NT processes, some events continue to remain ill-defined (Li et al., page 1, 2nd col., parag. under Progress in Nuclear Transfer)." This further supports the unpredictability in the art - if it would be routine experimentation to produce cloned animals, then one could expect that any donor cell could be successfully used to produce any species of animal. Such has not been found to be the case. Li et al. teach, "the low efficiency and abnormal development of cloned animals are mainly due to incomplete reprogramming and abnormal gene expression." Li et al., page 2, 1st col., 2nd full parag. Li et al. further state, "most cloned embryos have been observed to fail to develop to term, and some of the surviving cloned animals have shown abnormalities. The major cause may reside in faulty or incomplete epigenetic reprogramming of the donor nucleus, which affects the gene expression needed for every developmental stage of cloned embryos and offspring. Most cloned embryos lose their developmental abilities during pre-implantation and gastrulation. Moreover, the surviving adults often show abnormalities (Li et al., page 2, col. 1-2, bridging parag.)." McEvoy et al., 2003, Reprod. Supp., 61:167-182 support this unpredictability, citing that the production of NT-derived

ruminants is an inefficient process that generally fails to generate viable offspring. They suggest that after NT, fetal losses are due to significant developmental retardation and placental inadequacies, and state the following, "Indeed, the fact that losses can occur at all stages and in various guises, ranging from gross degeneration of preimplantation embryos to sudden post-natal death of apparently normal offspring, confirms that NT procedures are frequently responsible for fundamental and far-reaching disruption of developmental norms. Intuitively, it could hardly be otherwise, given that the reconstructed egg comprises a severely traumatized host cytoplasm fused to a donor cell (or nucleus) with which, to a greater or lesser extent, depending on its origin, it is virtually incompatible from the outset. Therefore, the more remarkable phenomenon is that, against the odds, NT sometimes results in the generation of viable offspring (McEvoy et al., emphasis added, page 173, 2nd and 3rd parags. under Nuclear Transfer Technology)." Therefore, NT transfer is clearly not a method that only requires routine experimentation in order to practice, but a complex method that is unpredictable at various stages, as evidenced by the cited art.

The claims further encompass cloning of primates, which is found to be unpredictable for specific reasons. Vogel, 2003, Science, 300: 225-227 teach that Rhesus monkey nuclear transfer (NT)-generated embryos seemed normal at their early stages but were unable to develop further when implanted into a surrogate mother. This was because the cells had the wrong number of chromosomes, and that this aneuploidy resulted in the abortion of the fetus. This

was found to also be the case with human NT embryos (Vogel, page 225, 1st col., 5th parag. to 2nd col., 1st parag.; also 2nd col., 4th parag.). Simerly et al., 2003, Science, 300: 297 teach that, "Primate NT appears to be challenged by stricter molecular requirements than in other animals ... With current approaches, NT to produce embryonic stem cells in nonhuman primates may prove difficult - and reproductive cloning unachievable." See p. 297, 3rd col., last sentence. As the state of the art evidences, NT in primates is unpredictable, and the instant specification fails to provide teachings to show that primate NT using the claimed methods would result in pluripotent mammalian cells, it would have required undue experimentation for one of skill in the art to make and use the claimed invention.

With regard to Applicant indicating that the phenotype of the melusin null mice closely mimics the clinical history of human hypertensive patients and this is a model of a human condition (Applicant's response, page 20), Applicant's argument is found persuasive and as such, the rejection as it applies to claims 40-42 is withdrawn. It is noted, that as stated above, claims 12-14 are rejected because nothing in the specification enables the claimed animals that exhibit the phenotypes in claims 12-14 without being induced with a hypertensive condition.

Thus, the claims remain rejected.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 8, 10, 11 are newly rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 8, 10, 11, use the phrase, "hypertensive condition is determined by...". It is unclear what this means. To aid in clarity, the claims may be modified by deleting the phrase, "determined by" or by substituting the word, "determined" with "induced."

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

It is noted that while a scope of enablement has been written, the claims as written, encompass transgenic animals that exhibit no phenotype (i.e., look wild type) and animals that exhibit any phenotype, including those unrelated to the disrupted gene of interest and those phenotypes that are not a model of any disease or disorder. It is noted that the only limitation of claim 1 is that the animal has an altered melusin gene expression.

Claims 1-6, 15, 16, 20-22, 24, 25 are newly rejected under 35 U.S.C. 103(a) as being unpatentable over Brancaccio et al., 1999, Journal of Biological Chemistry, 274: 29282-29288, see IDS, in view of Flatschart and Sogayar, 1999,

Brazilian Journal of Medical and Biological Research, 32:867-875, and in view of Capecchi, 1989, Trends in Genetics, 5: 70-76.

Brancaccio et al. teach in vitro studies that characterize melusin. Branchaccio et al. teach that melusin appears to play a crucial role during maturation and/or organization of muscle cells (Brancaccio et al., page 29287, bottom of 1st col. to 2nd col.). Brancaccio et al. teach the sequence of the mouse melusin gene (Brancaccio et al., Figure 1). While Brancaccio et al. teach in vitro characteristics of melusin, they do not teach in vivo characterization of melusin.

At the time of filing, Flatschart and Sogayar teach that the actual function of a variety of cDNAs and EST (expressed sequence tags) are unknown and that the function of these genes can be elucidated by 2 main approaches, namely overexpression and expression interference (Flatschart and Sogayar, abstract). One example of in vivo interference of gene expression is through the generation of "knockout" animals (Flatschart and Sogayar, page 872, 1st col., 5th parag.). While Flatschart and Sogayar envision making knockout animals which could be used as another way of elucidating gene function of melusin, they do not teach how to make knockout mice.

Capecchi teaches that through gene targeting, the potential now exists to generate mice of any desired genotype. The experimenter chooses both which gene to mutate and how to mutate it. Not only can gene targeting be used to generate null alleles, it can be used to modify any property of the gene that affects its function, such as it transcriptional pattern, its mRNA or protein maturation pattern, or the ability of the protein product to interact with other gene

products (Capecchi, page 70, 1st col., 3rd parag.). Capecchi teaches that generating knockout mice can be achieved by carrying out gene targeting in ES cells, using homologous recombination (Capecchi, Figure 3). Capecchi teaches that targeting vectors can be sequence replacement vectors and sequence insertion vectors. In both situations, the constructs comprise nucleic acid sequences that are homologous to endogenous sequences of the genome. Located between the homologous sequences in the construct are selection markers (Capecchi, Figure 3). As for methods drawn to making mice that are heterozygous and homozygous for the mutation (claims 24, 25), Capecchi teaches in Figure 2 that ES cells comprising the transgene construct are made in one genetic background (here, mice with black coats) and that the ES cells are injected into the blastocoel of mouse embryos that have another genetic background (here, mice with white coats). Following development in a pseudopregnant mother, chimeric mice are crossed with mice that are the same genetic background as the ES cell (mice with black coats). Of the mice with a targeted disruption in the gene of interest, the heterozygous disrupted mice are crossed with each other to obtain homozygous mice. As for obtaining the cells from the claimed mice (e.g. claim 20), it is well known that cell cultures from a host animal are made to quickly and more easily study biological activities in a cell.

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to make a transgenic mouse comprising a disruption in its melusin gene.

One having ordinary skill in the art would have been motivated to make a melusin knockout mouse in order to obtain an in vivo system of determining the biological role of melusin. Capecchi provides guidance on how to make knockout mice and Flatschart and Sogayar provide guidance that making knockout mice is one way of helping an artisan determine the function of a gene.

There would have been a reasonable expectation of success as Capecchi teach that gene targeting can be used to generate null alleles, that the method can be achieved using ES cells from mice, and that the targeting vector comprise sequence that is homologous to endogenous sequences of the genome.

Claims 1-4, 15, 20 are newly rejected under 35 U.S.C. 103(a) as being unpatentable over Brancaccio et al., 1999, Journal of Biological Chemistry, 274: 29282-29288 in view of Ignelzi et al., 1995, Crit. Rev. Oral Biol. Med. 6: 181-201.

Brancaccio et al. teach in vitro studies that characterize melusin. Branchaccio et al. teach that melusin appears to play a crucial role during maturation and/or organization of muscle cells (Brancaccio et al., page 29287, bottom of 1st col. to 2nd col.). Brancaccio et al. teach the sequence of the mouse melusin gene (Brancaccio et al., Figure 1). While Brancaccio et al. teach in vitro characteristics of melusin, they do not teach in vivo characterization of melusin.

At the time of filing, Ignelzi et al. teach that transgenic approaches are often used to gain insight into gene function. If an artisan wants to determine the effect that ectopic expression of a particular gene has on development in general, then expression of the gene of interest would be driven with a promoter

that has a wide spectrum of spatial and temporal expression. One example is a cytomegalovirus promoter, which has been shown to be active in a wide variety of mouse tissues throughout development (Ignelzi et al., page 182, 2nd col., 1st parag.). Ignelzi et al. teach that injection of altered genetic material into the pronucleus of a one-cell fertilized embryo (Fig. 1) has become the most commonly utilized technique to introduce foreign genetic material into the mouse germline and this technology has enabled artisans to gain insight into the expression and function of a multitude of gene (Ignelzi et al., page 182, parag. under "(1.1) Transgenic mice by pronuclear injection").

Therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to make a transgenic mouse that overexpresses melusin.

One having ordinary skill in the art would have been motivated to make a melusin overexpression mouse in order to obtain an in vivo system of determining the biological role of melusin, as Ignelzi et al. teach that making transgenic animals that overexpress a gene of interest (here, melusin) would give insight into the function of melusin.

There would have been a reasonable expectation of success as Ignelzi et al. teach that microinjection of an overexpression construct is routine in the art and that the technology has provided insight into the expression and function of a multitude of genes.

Conclusion

No claims allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Joanne Hama, Ph.D. whose telephone number is 571-272-2911. The examiner can normally be reached Monday through Thursday and alternate Fridays from 9:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, can be reached on 571-272-4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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